U. S. Department of Agriculture
Agricultural Research Administration
Bureau of Agricultural and Industrial Chemistry
Northern Regional Research Laboratory, Peoria, Illinois

PROCEEDINGS AND REPORT OF SEMINAR ON CHROMATOGRAPHIC METHODS

February 14-16, 1949

Contents

I. INTRODUCTION

The object of Dr. Hilbert in calling this meeting was to direct attention to the value of chromatography as a tool for the solution of problems encountered in the preparation, fractionation, purification, and identification of materials and compounds in chemical and biochemical research. To this end the knowledge and accumulated experience of experts throughout the Bureau were marshaled for the purpose of joint consideration and mutual exchange. Because of its closely allied nature, both in theory and in application, it was decided to include countercurrent distribution as a topic in the Seminar.

Participants in the Seminar included representatives from each of the four regional laboratories and three Bureau divisions. At the formal sessions on the first two days the total attendance varied from 45 to 65.

The program, comprising 16 reports, was organized around the subject: Carbohydrates, Oils and Lipids, Penicillin, Amino Acids, and Miscellaneous Compounds. Formal presentation of papers was followed by general discussion of methods, techniques, and apparatus. An exceptionally active interest in chromatography was apparent from the stimulating questions and discussions after each paper. Much thought has been given to the preparation of the report of this meeting in order to present the many factors involved in a form suitable for ready reference without sacrificing detail.

From the various types of work reported and the content of numerous review articles on chromatography, it may be concluded that there is hardly any class of chemical compounds to which the chromatographic approach cannot be applied. One significant lesson to be learned from the material presented is that progress in the application of this tool will not be hindered greatly by the need for development of new techniques and apparatus. While the necessity for developmental studies along specific lines may arise from time to time, much progress can be made in the solution of many of our problems simply by the application of the principles and methods now available.

If nothing else were gained from this meeting but the appreciation of the splendid advances in the work of the Bureau through the use of chromatography, the inspiration derived from these achievements alone would have justified the meeting.

II. ATTENDANCE LIST

Bureau of Agricultural and Industrial Chemistry

McCready, R. M.	Industrial Products Division, Western Regional Research Laboratory
Martin, L. F.	Head, Agricultural Chemical Research Division, New Orleans, Louisiana
Porter, W. L.	Analytical and Physical Chemistry Division, Eastern Regional Research Laboratory
Schaffer, P. S.	Biologically-Active Compounds Division, Beltsville, Maryland
Spies, J. R.	Allergen Research Division, Washington, D.C.
Swift, C. E.	Oil, Fat, and Protein Division, Southern Regional Research Laboratory

Northern Regional Research Laboratory

Milner, R. T., Director

Analytical and Physical Chemical Division

Earle, F. R.
Fett, Helen
McGuire, T. A.
Macmillan, Duncan
Melvin, E. H.
Senti, F. R.

Agricultural Residues Division

Aronovsky, S. I.
Nelson, G. H.
Russell, C. R.
Schniepp, L. E.

Commodity Development Division

Majors, K. R.

Engineering and Development Division

Anderson, R. A.
Beal, R. E.
Brekke, O. L.
Van Ermen, Louis
Zipf, R. L.

Fermentation Division

Benedict, R. G.
Koepsell, H. J.
Lockwood, L. B.
Nelson, G. E. N.
Riley, A. C.
Shotwell, O. L.
Stodola, F. H.
Tsuchiya, H. M.

Oil and Protein Division

Babcock, G. B.
Cannon, Joseph A.
Cowan, J. C.
Croston, C. B.
Dutton, H. J.
Evans, C. D.
Johnsen, V. L.
Kawahara, F. K.
Lancaster, C. R.
McKinney, L. L.
Scholfield, C. R.
Schwab, Arthur
Smith, A. K.
Teeter, H. M.
Uhing, E. H.

Starch and Dextrose Division

Alexander, B. H.
Cannon, John A.
Davis, H. A.
Dimler, R. J.
Hodge, J. E.
Jeanes, A. R.
Lohmar, R. L.
MacMasters, M. M.
Mehltretter, C. L.
Mellies, R. L.
Montgomery, E. M.
Weakley, F. B.
Wilham, C. A.
Wise, C. S.
Wolf, M. J.
Wolff, I. A.

III. PROGRAM

February 14 (9:00-12:00; 1:30-4:30)

Dr. R. T. Milner	Introduction
Mr. C. E. Rist, Chairman	Statement of the Object and Plan of the Seminar
	CARBOHYDRATES
Miss E. M. Montgomery	Fractionation and Identification of Polysaccharide Hydrolytic Products on a Carbon-Celite Column by Displacement Chromatography
Mr. F. B. Weakley	Laboratory Procedure for Chromatography Using Carbon-Celite Column
Dr. W. L. Porter	The Use of Paper Chromatography in the Determination of Glucose, Galactose, and Rhamnose in Mixtures
Dr. R. M. McCready	Application of Paper Partition Chromatography to Sugars
Dr. M. J. Wolf	Paper Chromatography of Some Mono- and Disaccharides
	PENICILLIN
Dr. F. H. Stodola	Chromatographic Separation of Penicillins

February 15 (8:30-12:00; 1:30-5:00)

	OILS AND LIPIDS
Dr. H. J. Dutton	Some Principles of Countercurrent Distribution
Mrs. C. R. Lancaster	Countercurrent Distribution Studies on the Chlorophyll and Carotene Pigments
Mr. C. R. Scholfield	Application of the Craig Apparatus to Soybean Phosphatide Fractionation Problems
Dr. H. J. Dutton	Comparison of Physical Methods of Fractionating Soybean Oil (including chromatographic and countercurrent extraction methods)
Dr. W. L. Porter	Quantitative Separation and Estimation of C_2 to C_{20} Fatty Acids in Mixtures
Mr. C. E. Swift	Applications of Chromatography to Separations of Minor Components of Fats and Oils
	OTHER COMPOUNDS
Dr. W. L. Porter	Application of Chromatography to the Separation of Miscellaneous Compounds (lactic acid, alcohols, aldehydes, amino acid derivatives, and pigments)

Dr. R. M. McCready	The Fractionation of Amino Acids Using Ion Exchange Resins
Dr. J. R. Spies	Applications of Chromatography at the Allergen Research Division
Dr. P. S. Schaffer	Applications of Chromatography at the Division of Biologically Active Chemical Compounds

February 16

A short meeting was held on the morning of this day for the discussion of conclusions and recommendations.

Representatives from other Laboratories and Bureau Divisions visited with laboratory personnel and inspected laboratory apparatus and equipment during the remainder of the day.

ACKNOWLEDGMENTS

Conference arrangements	Miss Louise Huntsinger
Preparation of seminar report	Dr. Allene Jeanes Dr. Fred K. Kawahara Mr. Joseph A. Cannon

IV. ABSTRACTS OF PAPERS PRESENTED

Fractionation and Identification of Polysaccharide Hydrolytic Products on a Carbon-Celite Column by Displacement Chromatography -- Edna M. Montgomery

Carbon-celite columns have been employed in chromatographic procedures using sorption and desorption techniques for the separation of the carbohydrate constituents of some enzymic and acid hydrolyzates of starch and starch products. The techniques were adapted from the researches of Tiselius. Analysis was obtained by displacement procedures of three types.

(1) Separation of mono- and disaccharides on columns of relatively large diameter. Capacity was increased by use of multiple columns since long columns cut down speed of process.

(2) Separation of sugars of like molecular weights, i.e., disaccharides, was carried out on columns of very small diameter.

(3) Partial separation of strongly adsorbing carbohydrates, i.e., dextrins, was accomplished by adsorbing on carbon partially saturated with the developer.

Fractionation was obtained by forcing an aqueous solution of the unknown through the column, by pressure or suction, in continuous flow. The column was kept under water at all times in the cylinder and the sugars were pushed off the column by a developer in the aqueous wash. This developer, or displacer, pushes the sugar off the column in the order of their increasing respective adsorptions. Fractions are tentatively characterized by means of a calculated $[\alpha]_D^{25^\circ}$ value obtained by use of the observed rotation and the concentration indicated by the copper reducing power. Absolute identification is made chemically.

Partial separation of α-malt-amylase dextrins of waxy corn starch and corn amylopectin, respectively, were each made by procedure (3). Industrial "hydrol" was analyzed by combining (1) and (2). Waxy corn starch and amylopectin mold amylase hydrolyzates were each analyzed by procedure (1). "Isomaltose," or the branching unit, a disaccharide designated 6-[α-D-glucopyranosyl]-D-glucose, was isolated in its predicted yield of 5 percent. The isolation is the first which has been made of this sugar by such a method and the first isolation of this sugar from starch.

Laboratory Procedure for Chromatography Using Carbon-Celite Columns -- F. B. Weakley

The laboratory procedure for the analysis of fungal amylase hydrolyzates of waxy corn starch and corn amylopectin is described in detail. The purification of the samples by means

of ion-exchange resins, the choice of developers, the assembly of the columns, the mixing of the adsorbent, the operation of the columns, and the characterization of the displaced fractions are described. The quantitative recovery in the different fractions of the applied carbohydrates and the high purity of the fractionated carbohydrates in the initial effluent solutions are features of the method.

The Use of Paper Chromatography in the Determination of Glucose, Galactose, and Rhamnose in Mixtures -- W. L. Porter and Charles S. Fenske

A method is presented for the analysis of mixtures of glucose, galactose, and rhamnose in the hydrolyzates of flavonol glycosides. To minimize the number of fermentations and the amount of quantitative analytical work, use is made of filter paper chromatography for qualitatively identifying the sugars present in the unknown solutions. A tank for use in preparing the chromatograms is presented. The sugar determinations are made by use of Schoorl's copper reduction method before and after fermentation by two yeasts capable of the selective destruction of glucose, and of glucose and galactose. /Unpublished/

Application of Paper Partition Chromatography to Sugars -- R. M. McCready

A description of apparatus and methods of separating sugar mixtures on paper sheet supports was presented. Whatman Numbers 1 and 4 filter paper was used. Quantities of 1 to 20 microliters of solution containing about 0.5 to 200 micrograms of sugar were separated. Phenol, n-butanol, collidine, lutidine, chloroform, benzene, and other solvents, either saturated with, or containing some water, served as moving solvents for the development of the paper partition chromatograms. Reagents sprayed on the paper sheets to reveal the positions occupied by the reducing sugars included ammoniacal silver nitrate, 3,5-dinitrosalicylate (prepared according to Sumner and Sisler, Archives of Biochemistry, 4, 333 (1944)), 0.1 percent resorcinol in 1 N hydrochloric acid for ketoses, and 0.1 percent naphthoresorcinol in 1 N hydrochloric acid for uronic acids.

Special techniques were discussed, including capillary ascent, two dimensional chromatography, and multiple development. The latter method is carried out by drying the developed chromatogram and repeating the procedure of humidification and development. This process of alternate drying and development may be carried out as many times as necessary for separation.

The results describing the movements of sugars are expressed in "R_G" values;

$$\frac{\text{distance of movement of unknown}}{\text{distance of movement of standard (glucose)}}$$

and "R_F" values;

$$\frac{\text{distance of movement of unknown}}{\text{distance of movement of solvent}}.$$

"R_G" indices are less affected by changes in temperature and solvent composition than are "R_F" values, and are probably more reliable.

The application of paper partition analysis to the problem of separating sugars from a hydrolyzate of purified pectic acid was described. Rhamnose, galactose, and arabinose were shown to occur in the ratio of 3:2:1. The carbohydrates of citrus juice were examined using paper partition analysis. Lemon, orange, and grapefruit juice contain fructose, glucose, and sucrose. No other sugars occur above a concentration of 0.05 percent. Application of the method to the separation of amino acids and organic acids was mentioned.

Recent reviews on this general subject include that of Consden (Nature 162, 359 (1948)) and Strain (Analytical Chemistry 21, 75 (1949)). /Unpublished/

Paper Chromatography of Mono- and Disaccharides -- M. J. Wolf

Qualitative analyses have been made of some carbohydrate constituents of the hull, germ, and endosperm of corn. The preliminary development of standard conditions and apparatus is described.

Three main developing solvents were employed: n-butyl alcohol (containing water and acetic acid), collidine saturated with water, and phenol saturated with water. Good separations were made between various pentoses, hexoses, and disaccharides if two or three of these solvents were used successively on the mixtures. Known pairs of closely related disaccharides, such as maltose and cellibiose, were not successfully separated by any combination of solvents employed. The limited work on solvent mixtures for the separation of closely related sugars which has been done thus far will be extended.

After evaporating the solvents, spots due to the sugars were detected by oxidation with ammoniacal silver nitrate or 3,5-dinitro salicylic acid. The latter reagent appears to be less sensitive, more specific for reducing sugars, and is only slightly affected by residues left in the paper after evaporating off the solvent. Ammoniacal silver nitrate is more sensitive and will detect not only reducing sugars, but alcohols, such as inositol, as well. It has the disadvantage of leaving a dark background with residues from solvents such as phenol.

Preliminary analyses of an acid hydrolyzate of corn pericarp showed that xylose, arabinose, and glucose were present. A fourth constituent of low R_f value has not as yet been identified. On acid hydrolysis, a hemicellulose preparation derived from corn pericarp also yielded xylose, arabinose, and glucose. Traces of one or possibly two additional substances remain to be identified. Glucose was the only carbohydrate constituent found in aqueous extracts of corn germ and endosperm, respectively, at 49° for two days. Acid hydrolysis of these extracts yielded no additional constituents. Because of some variability in R_f values in interpreting data, emphasis was placed on the use of two or three developing solvents and of known solutions of sugars to identify unknown components of a mixture, rather than on absolute measurements of R_f values. Analyses up to now indicate that the technique of paper partition chromatography provides a convenient and useful tool for the analyses of carbohydrate mixtures.

Chromatographic Separation of Penicillins -- Frank H. Stodola

The rapid development of the chemistry of penicillin was due, in large part, to the successful application of the principles and practices of chromatography. Early work was done with alumina columns, using first the free acid and later the sodium salts of penicillin. Because too much inactivation took place on this adsorbent, it was abandoned in favor of the silicagel-phosphate buffer column which was ideally suited for the resolution of the complex mixture of penicillins.

This technique is based on the method of Martin and Synge and is carried out as follows. Silica gel is impregnated with half its weight of concentrated phosphate buffer at pH 6.2. This produces an apparently dry powder which is slurried in wet chloroform. The column is formed from this slurry under gentle pressure and the slurrying solvent replaced by the desired solvent saturated with water. The penicillin is applied to this column as the free acid in ether, and the column is developed with the same solvent. Depending on their acid strengths, the various penicillins distribute themselves in bands throughout the column. Some of the more weakly acidic penicillins can even be washed off the column completely. The column is then extruded, sectioned, and the penicillin fractions removed by buffer solutions. Using this technique five natural penicillins have been obtained in the pure state.

Besides this major contribution to the problem, chromatography also aided materially in the (1) separation of the halogenated penicillins, (2) separation of the azopenicillins, (3) separation of the biosynthetic penicillins, (4) microanalysis of penicillin mixtures in broth, and (5) isolation of other acids associated with the penicillins.

Some Principles of Countercurrent Distribution -- Herbert J. Dutton

Distribution of solutes between immiscible solvents is not new as a means of separating mixtures; however, the apparatus developed by Craig for performing countercurrent distribution has made it possible to conduct tedious operations which are formidable, if not impractical, by the separatory funnel technique.

Important among the advantages of this method is the possibility of predicting the separation and behavior of solutes. Based upon the binominal theorm, rapid methods are developed for calculating partition coefficients and theoretical distribution curves. With the application of statistical theory, it is possible to predict the degree of separation that may be expected in any one of the available countercurrent distribution models or to calculate the number of transfers necessary to separate a given compound from another with a desired purity.

Countercurrent Distribution Studies on Chlorophyll and Carotene Pigments -- Catherine R. Lancaster

The countercurrent separation of a mixture of chlorophyll a, chlorophyll b, and carotene is described. With use of a 25-tube Craig distribution apparatus, an incomplete separation of the chlorophylls was obtained. Thus 14.95 percent of the chlorophyll a with a purity of 91.8 percent was isolated; similarly, 43.5 percent of the chlorophyll b with a purity of 91.0 percent was obtained. By contrast, 52.2 percent of the carotene was isolated with a purity of 98.7 percent.

The calculation of distribution curves for a 54-tube model indicate that its use would increase the fractionation of chlorophyll a to 68.5 percent, chlorophyll b to 70.0 percent, and carotene to 99.4 percent, with purities of 91.8 percent, 92.4 percent, and 99.0 percent, respectively.

A method for predicting the degree of separation between two solutes when partition coefficients and number of plates are known is demonstrated for the chlorophyll pigments.

Application of the Craig Apparatus to Soybean Phosphatide Fractionation Problems -- C. R. Scholfield

Soybean phosphatides were separated into portions soluble in absolute alcohol and insoluble in absolute alcohol. Each portion was then fractionated by distribution between immiscible solvents in the Craig countercurrent distribution apparatus. In the case of the alcohol-soluble phosphatides, hexane and 90 percent methanol were found to be suitable solvents. The fractions from each tube were weighed and

analyzed for phosphorous, choline nitrogen, amino nitrogen, and sugar, and the analytical values obtained were plotted against the tube number. Lecithin, cephalin, and a sugar-containing compound were shown to be present in this alcohol-soluble portion.

For the alcohol-insoluble phosphatides, hexane and 95 percent methanol were found to be suitable solvents. Phosphorous, total nitrogen, inositol, and sugar were determined on the fractions. At least two inositol-containing phosphatides were shown to be present. In addition, sugar-containing compounds were found.

The shapes of the distribution curves obtained deviated greatly from the theoretically calculated curves. However, useful separations were made, and valuable information concerning the composition of soybean phosphatides was obtained.

Comparison of Physical Methods of Fractionating Soybean Oil -- Herbert J. Dutton

Distillation, adsorption, distribution between immiscible solvents, and crystallization have been used to fractionate soybean oil. Columnar distillation procedures are generally inapplicable because of the short mean free path of glyceride molecules. Molecular distillations, unfortunately, are confined to single plate fractionations. While molecular distillation can be used effectively for stripping trace materials from the oil, such as sterols, tocopherols, pigments, etc., little fractionation of glycerides with respect to unsaturation can be effected.

Adsorption, liquid-liquid extraction, and crystallization techniques have been brought to bear upon the fundamental problem of determining the pattern of distribution of fat acids in the glycerides of soybean oil, whether even or random. As a result, comparisons of efficiency and applicability of the methods are made. On the basis of data obtained, it appears that the rule of even distribution in its elementary conception is not obeyed. Proof of the distribution pattern, whether random or otherwise, must await the further refinement of techniques. At the present time, separation of soybean oil into low iodine value edible fractions and high iodine value paint oils, appears limited by the efficiency of fractionation procedures rather than by the pattern of fat acid distribution.

Separation and Estimation of C_2 to C_{20} Fatty Acids in Mixtures -- W. L. Porter

Dr. C. L. Ogg has applied Patterson and Ramsey's published procedures to the identification and determination of the steam volatile fatty acids obtained by saponification of

Japanese buckwheat fat. By a combination of vacuum distillation from a buffered solution, steam distillation of the residue and chromatography on silicic acid, the volatile fatty acids were shown to be 98 percent lower fatty acids and 2 percent probably C_{12} or more. The low molecular weight fraction was found to consist of about 35 percent formic acid and 65 percent of an unidentified acid. The formic acid was identified qualitatively by its cerous salt and determined quantitatively by the A.O.A.C. $HgCl_2$ procedure (P 32.39, 6th Ed., 1945). The only other acid present was tentatively identified by the mercurous salt as butyric acid and determined by difference. [Unpublished]

Mr. R. W. Riemenschneider has obtained pure methyl linoleate and methyl linolenate from methyl esters of suitable starting oils by means of chromatographic adsorption, using silicic acid columns. Methyl linolenate having an iodine value within 1 percent of theory has been obtained in 35 percent yield. This was rechromatographed to give the pure compound. These studies are being continued in order to prepare pure methyl arachidonate from the methyl esters of the lipids of beef suprarenals.

The pure methyl esters isolated thus far have been used in studies of ultraviolet spectrophotometry. The specific extinction coefficient of alkali isomerized methyl linolenate prepared by the adsorption technique is significantly different from that of debromination linolenate. This has led to revised constants for use in the spectrophotometric determinations of linoleic and linolenic acids. [Presented before Am. Oil Chem. Soc. Meeting, Nov. 15-17, 1948]

Application of Chromatography to the Separation of Miscellaneous Compounds -- W. L. Porter

Lactic Acid.- The technique of Patterson and Ramsey for the chromatographic treatment of lactic acid has been employed by Dr. C. O. Willits and Dr. C. L. Ogg. The method has been used for testing the purity of lactic acid solutions that are obtained either directly or indirectly from fermentation processes. The results are used to substantiate the oxidation method which converts the lactic acid to acetaldehyde. Some indications have been found that so-called "pure" lactic acid is often either accompanied by a second organic acid or a small fraction of it may exist as a lactide. Either of these two explanations would account for low values reported in the literature as found by the oxidation method. [Unpublished]

Alcohols.- Dr. J. W. White, Jr., employed Brockmann's fluorescence techniques to the separation of the 3,5-dinitrobenzoates of the alcohols. The adsorbent was silicic acid-rhodamine 6 G and the developing solvent was ether-hexane. Twelve aliphatic alcohol (C_1 to C_6) dinitrobenzoates were studied. Of the 66 possible pairs, only 6 were completely inseparable, 11 were only partly separated and 49 were completely separated. [Unpublished]

Carbonyl Compounds.- Dr. White has also developed a method for the chromatography of the colored 2,4-dinitrophenylhydrazones of aldehydes and ketones. By adsorption on bentonite followed by hexane-ether development, four homologous methyl ketones, six homologous normal aldehydes (C_1 to C_6) and two isoaldehydes were separated in synthetic mixtures. None formed mixtures which were completely inseparable. [Anal. Chem. 20, 726 (1948)]

Amino Acids and Derivatives.- Dr. Sam Hoover and Dr. Edward Mellon have been using dinitrophenyl derivatives to study the end groups of proteins and peptides. They have found filter paper chromatography to give highly reproducible results and have been able to separate in a satisfactory manner all the dinitrophenyl amino acids except the following combinations: proline-alanine, phenyl alanine-leucine-isoleucine, and glutamic acid-aspartic acid. Both ascending and descending solvent methods are used. The ascending method has greater ease of operation but in some cases does not give as perfect separation as does the descending solvent method on longer strips. The solvents employed and found most satisfactory are: n-butanol (50 percent), water (50 percent); n-butanol (16 percent) n-butylacetate (34 percent), 50 percent of 1 percent NH_4OH; phenol (50 percent), water (50 percent); collidine (25 percent), chloroform (25 percent), water (50 percent); benzene (50 percent), 1 percent acetic acid (50 percent). These dinitrophenyl derivatives are colored yellow and their spots are easily visible on the paper. Since no reagent is needed to mark the spot, it is a simple matter to elute a spot obtained from a mixture and to study it further on different chromatograms until its identity is determined and proven. In the paper chromatography of amino acids, Drs. Hoover and Mellon found that the intensity of the amino acid-ninhydrin color on the paper may be increased by exposing the paper, after development, to the effects of live steam. Sometimes an additional intensification will occur on repeating the development. By this method as little as 0.1 microgram of amino acid can be detected. This corresponds to about one microgram per milliliter. [Unpublished]

Tobacco Alkaloids.- Dr. Abner Eisner has been able to separate myosmine from nornicotine using a silicic acid column. The eluant was 7 percent acetone in Skellysolve B. Myosmine being less strongly adsorbed was eluted first, followed about 1 cm. later by the nornicotine. The column was about 1 cm. in diameter. Elution progress was followed by the fluorescence produced by exposure of the column to ultraviolet light. The fractions of the eluate were examined spectrophotometrically for qualitative identification of the components of the mixture. [Unpublished]

Carotene.- Dr. Edward G. Kelley and Dr. Monroe E. Wall have published a quantitative method for the estimation of carotene in plant tissues and extracts. A column consisting of 3 parts HiFlo Supercel and 1 part of activated magnesia is used to separate carotene from xanthophyll and chlorophyll. Adsorption is from hexane and elution is with 3-5 percent acetone in hexane. Spectrophotometric analysis of the eluate is the final step. [Ind. Eng. Chem., Anal. Ed. 15, 18 (1943)]

Tocopherol.- Using the same adsorbent and solvents, they separated tocopherol from xanthophyll and chlorophyll. The tocopherol eluate was freed from carotene by treatment with sulfuric acid and either of two methods (Furter-Meyer or Emmerie-Engel) is employed for the quantitative estimation. [Ind. Eng. Chem., Anal. Ed. 18, 198 (1946)]

Leaf Sterols.- Drs. Kelley and Wall have separated the leaf sterols and carotene from xanthophyll and chlorophyll by the same chromatographic procedure and after precipitation of the carotene as the insoluble iodide, have determined the sterols spectrophotometrically after reaction with digitonin. [Anal. Chem. 19, 677 (1947)]

Vitamin A Ester.- The same adsorbents and solvents were employed as for carotene, tocopherol and the sterols. However, in this instance, both size of column (7 cm. x 2.5 cm.) and the volume of the eluant (25 ml.) are critical, since under these conditions carotene is not eluted but vitamin A ester is removed. [Anal. Chem. 20, 757 (1948)]

Chlorophyll.- Preparative Method by Dr. Wall. Chlorophyll extracts in hexane, containing about 10 percent chlorophyll, are passed through long porocel (activated bauxite) columns. Four grams of chlorophyll in 40 gms. of solids were adsorbed on a 36" x 1" column containing 600 grams of porocel. The column was washed with hexane and with 20 percent hexane in acetone. Finally the chlorophyll was eluted with methanol. The yield was 80 percent chlorophyll with a purity of 50 percent. The eluted chlorophyll was then chromatographed on sucrose and chlorophylls a and b were partially separated. [Unpublished]

Riboflavin.- Dr. Kelley has developed a method for the isolation of riboflavin. The H_2SO_4 extract of the plant material is filtered and the riboflavin adsorbed on Florosil (Super-Sorb) held in a 1 cm. diameter tube having a depth of 6 inches of adsorbent. After washing with cold and hot water, the adsorbed riboflavin is eluted with pyridine-acetic acid according to the method of Conner and Straub. The eluate is then oxidized with permanganate to remove impurities and the riboflavin determined fluorimetrically. /Unpublished/

Red Pigment from Buckwheat.- Dr. W. L. Porter has developed a tentative method for the isolation of the factor in buckwheat which causes fagopyrism. Buckwheat fat was dissolved in 80 percent ethanol, the mixture extracted with Skellysolve B, and then with chloroform after adjusting the ethanol concentration to 60 percent. The chloroform extract was dried with Na_2SO_4 and evaporated to dryness. The residue, dissolved in 95 percent ethanol, was adsorbed on Florosil and washed with ethanol, then chloroform-ethanol (50-50), and then with 5 percent acetic acid. When the acetic acid eluate was clear, the pigment was eluted with 5-10 percent hydrochloric acid. The hydrochloric acid eluate was extracted with chloroform and after being washed free of acid with water, drying with Na_2SO_4, and evaporated to dryness, a residue was obtained which gave a specific extinction coefficient of 5. The curve shape compared almost exactly with the absorption curve for hypericin from St. John's Wort which had a specific extinction coefficient of 25. However, it is believed that some impurity is still present in the buckwheat pigment since it has a waxy appearance and gives a low extinction value. These preparations are now being investigated pharmacologically. /Unpublished/

Separation of Carbohydrates, Amino Acids, and Organic Acids by Means of Ion Exchange Resins.- In the Maple Products project, maple sirup is being separated into three fractions according to the method of McKinney, et al. Nalcite HCR is used in the hydrogen cycle to remove amino acids, Duolite A-4 is used to remove the organic acids, and the sugars are non-adsorbed. These fractions will be used for studying the mechanism of flavor and color development in maple sirup production.

Applications of Chromatography to Separations of Minor Components of Fats and Oils -- C. E. Swift

Minor components of refined peanut oil were concentrated by molecular distillation and solvent fractionation and separated into fractions by chromatography on alumina and silicic acid. Low-boiling petroleum naphtha was used as initial solvent and for the development of chromatograms. The fractions obtained consisted of chroman-5,6-quinones, tocopherols, and two

pro-oxidants. The pro- and antioxygenic activity of the fractions was determined by adding them to lard or a special peanut oil substrate. This substrate consisted of a colorless antioxidant-free oil prepared by passing a solution of refined peanut oil in petroleum naphtha through a column containing stratified alumina, carbon, and activated clay. The chroman-5, 6-quinone was identified by comparison with a synthetic sample prepared by the chromatographic separation on silicic acid of the products obtained by oxidizing alpha-tocopherol with nitric acid.

A red pigment in crude rice bran oil was concentrated by adsorption on alumina and purified by chromatographic separation on silicic acid. The compound had some of the properties of an anthocyanin and exhibited properties of a pro-oxidant when added to lard. The pigment is present in crude, refined, and in some samples of bleached oils, as shown in their absorption spectra in which maxima occur at ca. 478, 488, 546, and 559mμ.

Aldehydes produced during the autoxidation of cottonseed oil were concentrated by steam distillation and converted into 2,4-dinitrophenylhydrazones. The mixture of 2,4-dinitrophenylhydrazones was fractionated on a column of alumina using petroleum naphtha-benzene (1:1) as initial solvent and to develop the chromatogram. The three principal bands yielded relatively pure fractions of the 2,4-dinitrophenylhydrazones of 2,4-decadienal, 2-octenal and hexanal.

Fractionation of Amino Acids Using Ion Exchange Resins -- R. M. McCready

General properties of five synthetic ion exchange materials were discussed.

A method suitable for the separation of amino acids into groups based on the work of Tiselius, Drake and Hagdahl (Experientia, 3, 21-26 (1947)) was described. The method utilized columns of charcoal for absorption of aromatic amino acids, carboxyl ion exchangers for absorption of basic amino acids, and sulfonic acid exchangers for absorption of the remaining acidic and neutral amino acids. These amino acids were further separated by elution from the sulfonic acid resin, evaporated, and passed through Amberlite IR-4.HAC, an anion exchange material. The acidic amino acids were absorbed and the neutral ones passed through unchanged. Elution of the amino acids from the charcoal absorbent was accomplished with phenol solution. The absorbed amino acids on the other exchange materials were eluted with hydrochloric acid. Upon evaporation of the eluates the separate groups of amino acids are obtained.

Preliminary work by Dr. Howard Walker at the Western Laboratory on sugar beet juice showed that the amino acids absorbed from beet juice by Dowex 30 (a strong acid cation exchange resin) are eluted with hydrochloric acid in the order of aspartic acid, glutamic acid, alanine, and a group consisting of valine, isoleucine and others. Careful control of acidity and flow rate may aid in attaining sharper separations of amino acids by this procedure. [Unpublished]

Applications of Chromatography at the Division of Biologically Active Chemical Compounds -- P. S. Schaffer

Chromatography has been employed on a small and experimental, or probing, scale in order to resolve natural plant growth regulators and plant antibiotics. A small quantity of crystalline material was separated from corn pollen with the use of an Al_2O_3 column. Other substrates that were tried were potato starch. In the plant antibiotic field we were able to separate at least three fractions of a plant extract with the use of a charcoal column, using solvents at different pH to effect the separation. Al_2O_3 has also been used with some success.

Applications of Chromatography at the Allergen Research Division -- J. R. Spies

An application of chromatographic adsorption of an allergenic natural protease picrate from cottonseed was described. The objectives of this study were both fractionation and test of homogeneity. The picrate was dissolved in either 50 percent aqueous ethanol or 50 percent dioxane. Quantities ranging from 1/2 to 20 grams in 0.5 percent solution were used. When such solutions were passed through a column of Brockmann's aluminum oxide, the picrate was adsorbed in a uniform yellow layer. The filtrate was allergenically inactive, but the eluted picrate diluted one to one million reacted positively on cottonseed sensitive subjects. Development of the column with the solvent caused resolution into two layers, but the two principle layers were not homogeneous by other criterion. [J.A.C.S. 62, 1420 (1940)]

V. CLASSIFICATION OF CHROMATOGRAPHIC STUDIES

Organization of the material presented in the 16 papers was deemed essential from the standpoint of obtaining a proper perspective and ready-reference tabulation for future consultation. Presented below is first a general classification scheme, with the expectation that it will be of value in three ways, viz., (a) to state concisely the methods, techniques, and objectives commonly encountered; (b) to provide a scheme for the pictorialization of the studies reported at the Seminar; and (c) to serve as a form for the exchange of information on new developments and applications if and when it is decided to bring the Bureau's work in this field up to date periodically. Following the general classification outline are abstracts of the material presented in the several papers at the Seminar, arranged according to this general classification scheme.

Materials	Method	Technique	Objectives
1. Substrate 2. Name of reporter(a)	A. Adsorption 1. Displacement-development 2. Elution analysis 3. Frontal analysis 4. Extrusion of adsorbent column B. Partition 1. Paper a. Ascending b. Descending 2. Columnar 3. Countercurrent distribution C. Ion exchange etc. (Include key references here)	1. Apparatus 2. Adsorbent--support 3. Solvents--solutions--eluants 4. Method of fraction analysis a. Spot development b. Spot elution c. Polarimetric d. Spectrophotometric e. Ultra-violet light--fluorescence f. Index of refraction g. Differential density h. Weight curve i. Internal indicator j. Chemical assay (1) Reducing power	1. Exploratory 2. Fractionation--resolution of mixtures; quantitative 3. Preparation--isolation 4. Proof of identity 5. Criterion of purity or homogeneity 6. Purification--removal of trace constituents 7. Concentration of one constituent 8. Scale 9. Decomposition 10. Quantitative determination etc.

Technique

(2) Color test
(3) Crystallization
k. Microbiological assay
l. Biological assay
m. Photo development (radioactive tracers)
n. Melting point

(a) Under the "Materials" column of this report the name of the person reporting the work is given.

* Critical Factor

** Research went into development

Future Reports

In the case that this scheme is adopted for the future periodic exchange of chromatographic developments and applications, the author's name could appear under (a), points critical to the success of the method could be marked *, and points reflecting the author's own development could be marked **.

Classification of Reported Work

Materials	Method	Technique	Objectives
Mono- and di-saccharides and dextrins from enzymic hydrolyzates of amylo-pectin and waxy corn starch; hydrol. Montgomery and Weakley	Adsorption, displacement-development. See: A. Tiselius and L. Hahn, Kolloid Z. 105, 177 (1943).	Apparatus: Three columns (30 cm. x 7.5 cm.) in series. Adsorbent: 1:1 mixture by volume of carbon (Darco G-60) and Celite 501. Solutions for displacement: Aqueous phenol and aqueous ephedrine. Method of fraction analysis: Polari-reducing power, crystallization.	Quantitative fractionation for isolation on a large scale.
Glucose, galactose, and rhamnose in acid hydrolyzates of flavonol glycosides from buckwheat. Porter	Partition, paper; descending type on sheets of paper.	Apparatus: Tightly-sealed wooden tank lined with stainless steel and having double-pane* glass windows for development; convection oven and box with light bulbs or resistance heater and electric fan for solvent removal. Solvent: Butanol-ethanol-water. Spray: Ammoniacal silver nitrate. Method of fraction analysis: Spot development. Reducing power and microbiological assays were run, also	Resolution of mixtures, proof of identity.

Materials	Method	Technique	Objectives
Hexoses and sucrose from citrus juice; hexoses, pentoses, methyl pentoses and sucrose from acid hydrolyzates of purified pectic acid. McCready	Partition, paper; descending type on sheets of paper.	Apparatus: Closed crock for developing, convection oven for drying. Solvents: Butanol-acetic acid-water, and others. Spray: Ammoniacal silver nitrate, 3,5-dinitrosalicylic acid, resorcinol or naphthoresorcinol in hydrochloric acid. Method of fraction analysis: Spot elution, spot development, reducing power, crystallization.	Quantitative resolution of mixtures on a micro-scale, proof of identity.
Pentoses and hexoses from acid hydrolyzate of corn pericarp and corn pericarp hemicellulose; glucose from aqueous extracts of corn germ and endosperm; di- and higher saccharides from acid and enzymic hydrolyzates of polysaccharides. Wolf	Partition, paper ascending and descending types on sheets and strips of paper.	Apparatus: Tightly closed glass jars protected against temperature changes,* for developing. Solvents: Butanol-acetic acid-water, collidine-water, phenol-water, and others. Spray: Ammoniacal silver nitrate, 3,5-dinitrosalicylic acid. Method of fraction analysis: Spot development.	Exploratory, resolution of mixtures and proof of identity on a micro-scale.

Materials	Method	Technique	Objectives
Penicillins and their derivatives Stodola	Adsorption, elution analysis and extrusion of adsorbent column.	Adsorbents: Alumina; silica gel impregnated with phosphate buffer. Method of fraction analysis: Microbiological assay, crystallization.	Fractionation; isolation on a micro- and industrial scale; criterion of purity.
Soybean oil. Dutton	Partition; countercurrent distribution. References: Craig, L. C., J. Biol. Chem., 168, 687 (1947). Martin, A.J.P., and Synge, R.L.M., Biochem. J., 35, 1358 (1941). Craig, L. C., J. Biol. Chem., 155, 519 (1944).	Apparatus: Craig machine.	Exploratory; fractionation.
Chlorophyll and carotene pigments. Lancaster	Partition; countercurrent distribution.	Apparatus: Craig countercurrent distribution apparatus. Solvents: Hexane and 90% ethanol. Method of fraction analysis: Spectrophotometry, weight curve.	Fractionation, exploratory.
Soybean phosphatides. Scholfield	Partition; countercurrent distribution. References: Scholfield, C. R., Dutton, H. J., Tanner, F. W., and Cowan, J. C., J.A.O.C.S., XXV, No. 10, 368 (1948).	Apparatus: Craig machine. Solvents: Hexane and 90% methanol, hexane and 95% methanol.	Resolution of mixtures.

Materials	Method	Technique	Objectives
Fatty acids from saponified Japanese buckwheat fat; methyl esters of oils and lipids. Porter	Partition, columnar. References: Ramsey, L. L., and Patterson, W. C., A.O.A.C., 31, No. 2, 441 (1948); ibid., 31, 139 (1948).	Support: Mallinckrodt silicic acid.* Solvents: Furfuryl alcohol, 2-aminopyridine as immobile solvent; n-hexane as mobile solvent. Also HOAC, NH_4OH, water. Method of fraction analysis: Chemical assay, spectrophotometric, internal indicator.	Quantitative fractionation, isolation, and purification.
Lactic acid. Porter	Partition, columnar. See references by Patterson and Ramsey.	Support: Mallinckrodt silicic acid. Solvent: Butanol, chloroform and water. Method of fraction analysis: Chemical assay.	Criterion of purity.
Alcohols as 3,5-dinitrobenzoates. Porter	Adsorption	Adsorbent: Silicic acid-rhodamine 6G. Solvent: Ether-hexane. Method of fraction analysis: Fluorescence.	Fractionation
Carbonyl compounds as 2,4-dinitrophenylhydrazones; volatile components of apple juice. Porter	Adsorption, extrusion of adsorbent column. Anal. Chem., 20, 726 (1948).	Adsorbent: Bentonite. Solvents: Hexane-ether. Method of fraction analysis: Melting point.	Fractionation, isolation.

Materials	Method	Technique	Objectives
Amino acids as dinitrophenyls. Porter	Partition, paper, ascending and descending.	Solvents: n-butanol and water; n-butanol, n-butyl acetate, and NH_4OH; phenol and water; collidine, chloroform and water; benzene and acetic acid. Method of fraction analysis: Spot elution.	Fractionation in micro amount; identification.
Tobacco alkaloids, myosmine and nornicotine. Porter	Adsorption, elution analysis.	Adsorbent: Silicic acid. Eluant: Acetone (7%) and Skellysolve B. Method of fraction analysis: Spectrophotometric fluorescence.	Fractionation
Carotenes in plant tissues and extracts. Porter	Adsorption, elution analysis. Ind. Eng. Chem., Anal. Ed., 15, 18 (1943).	Adsorbent: Hiflo Supercel, and activated magnesia. Solvent: Hexane. Eluant: Acetone (5%) and hexane. Method of fraction analysis: Spectrophotometric.	Quantitative determination.
Tocopherols in plant tissues. Porter	Adsorption, elution analysis. Ind. Eng. Chem., Anal. Ed., 18, 198 (1946).	Adsorbent: Hiflo Supercel and activated magnesia. Solvent: Hexane. Eluant: Acetone (5%) and hexane. Method of fraction analysis: (Furter-Meyer or Emmerie-Engel spectrophotometric).	Fractionation, quantitative determination.

Materials	Method	Technique	Objectives
Leaf sterols. Porter	Adsorption, elution analysis. Ind. Eng. Chem., Anal. Ed., 19, 677 (1947).	Adsorbent: Hiflo Supercel and activated magnesia. Solvent: Hexane. Eluant: Acetone (5%) and hexane. Method of fraction analysis: Spectrophotometric, chemical assay.	Fractionation, quantitative determination.
Vitamin A ester from poultry mash. Porter	Adsorption, elution analysis. Anal. Chem., 20, 757 (1948).	Apparatus: Column 7 x 2.5 cm.* Adsorbent: Same as for carotene, tocopherol and sterols. Solvent: Same as for carotene, tocopherols and sterols. Volume, 25 ml.* Method of fraction analysis: Electrophotometric analysis of glycerol dichlorohydrin reaction product.	Fractionation
Chlorophyll from plant extracts. Porter	Adsorption, elution analysis.	Apparatus: Column 36" x 1". Adsorbent: Porocel; sucrose. Solvent: Hexane; hexane (20%) and acetone. Eluant: Methanol. Method of fraction analysis: Spectrophotometric.	Fractionation, purification.
Riboflavin from plant extract. Porter	Column adsorption, elution analysis.	Apparatus: Column 1 cm. x 6 inches. Adsorbent: Florosil. Solvent: H_2SO_4. Eluant: Pyridine-acetic acid.. Method of fraction analysis: Fluorescence.	Isolation, quantitative determination.

Materials	Method	Technique	Objectives
Red pigment from buckwheat. Porter	Column adsorption, elution analysis.	Adsorbent: Florosil Solvent: 95% ethanol, ethanol, chloroform-ethanol (50-50), 5% acetic. Eluant: 10% HCl. Method of fraction analysis: Spectro-photometric.	Fractionation, isolation.
Carbohy-drates, amino acids, organic acids. Porter	Ion exchange	Adsorbents: Nalcit HCR, Duolite A-4.	Fractionation, isolation.
Hydrocarbon solvents for spectroscopy. Swift	Adsorption	Adsorbent: Silica gel.	Purification
Resin acids Swift	Adsorption	Adsorbent: Fuller's earth.	Purification (from phenols).
Soaps used in polymeri-zation of rubber. Swift	Adsorption	Adsorbent: Fuller's earth.	Purification
Picrate deriva-tives of poly-nuclear aromatics. Swift	Adsorption	Adsorbent: Alumina	Decomposition for recovery of aromatics.

Materials	Method	Technique	Objectives
Gossypol pigment from cottonseed Swift	Adsorption	Adsorbent: Starch and inulin.	Fractionation
Tocopherols, carotenes, sterols. Swift	Adsorption	Adsorbent: Inulin and silica.	Fractionation, quantitative determination.
C_1-C_5 acids. Swift	Adsorption	Adsorbent: Silica	Fractionation
Minor components of peanut oil. Swift	Adsorption, extrusion of adsorbent column.	Adsorbent: Alumina and silicic acid. Solvent: Low boiling petroleum naphtha.	Qualitative fractionation.
Red pigment from rice bran oil. Swift	Adsorption, extrusion of adsorbent column.	Adsorbent: Alumina silicic acid and Highflo Supercel.	Concentration, purification.
Products of auto-oxidation of cottonseed oil. Swift	Adsorption	Adsorbent: Alumina. Solvent: Petroleum naphtha-benzene (1:1).	Fractionation
Riboflavin and thiamin. Swift	Adsorption	Adsorbent: Decalso, Fuller's earth.	Resolution of mixture.

Materials	Method	Technique	Objectives
Piperonyl butoxide (contaminant in flour from cotton bag). Swift	Adsorption	Adsorbent: Silica	Concentration
Degradation products of cellulose. Swift	Adsorption	Adsorbent: Florex XXX	Fractionation
para-Cymene and menthane Swift	Adsorption	Adsorbent: Silica gel	Fractionation
Xanthophyll and carotenoides. Swift	Adsorption	Adsorbent: Magnesium oxide and calcium acid phosphate.	Fractionation
Acidic, basic, neutral, and aromatic amino acids; amino acids from sugar beet juice. McCready	Adsorption, elution analysis; ion exchange. See: The Resinous Reporter, July 1948; Kumin, Anal. Chem., 21, 87 (1949).	Adsorbents: Carbon, cation exchangers (sulfonic acid and carboxylic types), anion exchangers. Eluants: 5% phenol for carbon; dilute HCl for exchange materials.	Exploratory; small-scale fractionation.

Materials	Method	Technique	Objectives
Plant growth regulators; antibiotics from sweet-potato and tomato plants and from banana skins. Schaffer	Adsorption; partition on paper.	Adsorbents: Al_2O_3, carbon, potato starch, $CaCO_3$. Method of fraction analysis: Crystallization, biological and microbiological assay.	Exploratory, resolution of mixtures on a small scale.
Allergenic natural protease picrate from cottonseed. Spies	Adsorption, elution analysis.	Adsorbent: Brockmann's Al_2O_3. Solvents: 50% dioxane, 50% aqueous ethanol. Method of fraction analysis: Biological assay.	Fractionation on a small and medium sized scale; test for homogeneity.

VI. SUMMARY OF REPORTS AND DISCUSSIONS

The object of this section of the Seminar Report is to present all pertinent information which was reported at the Seminar without repeating statements made in Part IV, "Abstracts of Papers Presented." Special attention has been given to the inclusion of details regarding quantities, conditions, techniques, and apparatus. The material which follows is the interpretation by the several recorders and may not always represent the exact statements or opinions of the authors.

Fractionation and Identification of Polysaccharide Hydrolytic Products on a Carbon-Celite Column by Displacement Chromatography -- Edna M. Montgomery

The adsorbent used was carbon (Darco G-60) mixed with an equal part of Celite 501, which was non-adsorbing but served to increase the ease of liquid flow through the columns. Carbon was selected as the adsorbent because much is known about its adsorbing characteristics, it adsorbs well from aqueous solutions, it can be obtained commercially in relatively pure form, and Tiselius and coworkers have established the principles for its use in quantitative chromatographic work.

The maximum amounts of carbohydrates worked with were the hydrolyzates from 200 g. of waxy corn starch, and 300 g. of hydrol. When used on this scale, the recovery from hydrol was complete; when hydrol was worked with in quantities of 50 g., the loss was 5 to 8 percent.

Enzymic Hydrolyzate of Waxy Corn Starch.- Waxy corn starch, which is practically 100 percent amylopectin, was hydrolyzed to completion by a commercial enzyme preparation from Aspergillus oryzae. The hydrolyzate was made free from protein and mineral matter, and added to the adsorption column in aqueous solution. The constituents expected in the hydrolyzate were glucose, isomaltose, and dextrins. At pH 5.5 glucose is not adsorbed, but comes through the column as a "free zone." At pH 7.5-8.0 glucose is adsorbed, but can be washed off with water. Isomaltose was displaced by 0.5 percent aqueous phenol, and the dextrins were displaced by use of aqueous phenol in concentrations above 0.5 percent and up to 3.5 percent.

Investigation is now under way on the separation on carbon-Celite columns of the dextrins resulting from the successive action on waxy corn starch of β-amylase and α-malt amylase. The columns have been pretreated with 0.25 percent ephedrine.

Hydrol.- It has been estimated from a number of investigations on methylated and propionylated hydrol that hydrol contains approximately 1.8 percent hydroxymethylfurfural (HMF),

55-60 percent glucose, 5 percent gentiobiose, 25 percent isomaltose, 5.5-7.5 percent tri- and tetrasaccharides. In the work reported, glucose was separated from disaccharides on the large columns, and the fractionation of the disaccharides was carried out on small columns (1 cm. in diameter) by use of 0.5 percent aqueous phenol. The disaccharides were displaced from the column in the order: trehalose, isomaltose, an unknown substance, and gentiobiose.

The composition of hydrol, in percent, as found by fractionation on carbon-Celite columns was: hydroxymethylfurfural 1-2, glucose 70, trehalose 1.5, isomaltose 15, gentiobiose 5, tri- and tetrasaccharides 5, and small amounts of unknown substances.

Discussion

Question: In work with antibiotics, carbons differ greatly in adsorbing and selective power. Would the same results be obtained in your work by use of other carbons?

Answer: The carbon used was selected because of its large surface and high purity as obtained commercially. No other carbons have been tested.

Question: Have you isolated levoglucosan from enzymic hydrolyzates of starch?

Answer: Originally, yes. A maltosan has been isolated since, but not characterized.

Question: Was any difficulty encountered in concentrating effluent solutions from the columns for weight analysis?

Answer: Aliquots were not concentrated to dryness for weight analysis.

It was pointed out in ensuing discussion that Craig checks on the course of fractionation by concentrating aliquots and weighing on a microbalance. In work with numerous types of substances, dry weight curves add much information on the course of fractionation. For instance, one might be "all wrong" by relying on tests by fluorescence or refractive index, and could be "kept straight" only by use of weight curves.

Reducing power and optical rotation are peculiarly suitable for analysis of carbohydrates, especially when the composition is, in general, known. With higher molecular weight substances, such as dextrins, these methods will have to be checked more frequently by making dry-weight determinations of aliquots.

Question: Does mutarotation interfere with use of optical rotation for analysis?

Answer: Solutions were allowed to stand before readings were made.

Question: How has phenol been removed from the effluents?

Answer: It has not, because it does not interfere with Munson-Walker sugar determinations. When necessary, it could be extracted or steam-distilled. Ephedrine was extracted with chloroform or ethylene dichloride.

It was pointed out in subsequent discussion that phenol would interfere with sugar determinations by the Somogyi or Shaffer-Hartmann procedures.

Laboratory Procedure for Chromatography Using Carbon-Celite Columns -- F. B. Weakley

This report continued the description of the investigation reported by the preceding speaker.

The acid and enzymic hydrolyzates, before being applied to the carbon-Celite columns, were treated with basic lead acetate to deproteinize, and then passed, alternately, through two columns each of Amberlite IR-100 and IR-4 to demineralize.

Preparation of Columns for Use.- Each tube (30 cm. x 7.5 cm.) of the multiple column was prepared for use separately. The tubes had fritted glass plates at the base and these were carefully covered with a pad of glass wool before the adsorbent was added to the tube.

The components of the adsorption mixture, equal parts by volume of Darco G-60 and Celite 501, were thoroughly mixed in the dry state by mechanical agitation for 30 to 45 minutes with a rotary spinner. The total charge for the three tubes was mixed at one time, and then made to a very dilute slurry with water. For filling, a tube was placed on a suction flask, suction was applied, the pad of glass wool was moistened with water and pressed down with a rubber plunger, and then the slurry was added rapidly. Slow addition tends to give a bed with an uneven base. The depth of the bed was made to about 22 cm., a pad of glass wool was placed over the surface, and the charged tube was conditioned by continuous passage of distilled water. If the tube was packed with the dry adsorbent mixture, the bed tended to shrink when liquid was added to it. Slurrying the carbon and Celite separately before mixing was not satisfactory because Celite floated to the surface.

When not in use, the adsorbent columns were kept covered with water.

Regeneration of Adsorbent Columns.- After a run, the adsorbent column was made ready for use again by passing methanol through it, and then washing with water. The methanol removed phenol and any traces of protein that might be present. Regeneration in this way permitted a column, when once prepared, to be used five or six times, or until it cracked or pulled away from the wall of the tube.

Operation of Adsorption Column.- Solution or developer was forced under pressure from a storage container on to the column. Solutions were pulled through the columns with gentle suction at the rate of 1 to 1.5 liters per hour. The effluent was collected in a large suction flask from which aliquots were siphoned out by suction in such a way as not to change the pressure on the column.

Method of Analysis.- The reducing power of the carbohydrate solution was measured before it was put on the column. Desorption was followed by analysis of each liter of effluent. The carbohydrate content of the effluent was tentatively characterized by measurement of optical rotation, using a 4-dm. polariscope tube, and by determination of reducing power by the Munson-Walker method. From the measured rotations and the reducing values a specific rotation was calculated assuming the unknown to be maltose hydrate. This allowed the samples of different sugars to be detected readily. Fractions were identified conclusively by crystallization from concentrates of the effluent.

From the enzymic hydrolyzate of 200 g. of waxy corn starch there was recovered on the basis of total carbohydrate reducing value, calculated as glucose, glucose 92.6 percent, isomaltose 3.8 percent, and dextrins 3.5 percent.

Discussion

Question: Why was a column consisting of three tubes of equal size used?

Answer: By so doing high adsorptive capacity was obtained without the disadvantages of troublesome shrinking and slowness of flow that characterize larger single beds of adsorbents.

Question: Does mixing of solvent fronts occur when the solution accumulates at the top of the second and third tubes?

Answer: No pool of liquid accumulates at the tops of the columns because the rate of flowing in and out of the columns is constant. It is believed the fronts reform in each successive column.

It was brought out in ensuing discussion that Claesson has described chromatographic columns designed to cause mixing as the effluent from one column enters the top of the next one. This results in cleaner fractionation.

Question: Can isomaltose be crystallized directly after being removed from the column?

Answer: Yes, the same product is obtained as that from the deacetylation of the octaacetate.

Question: How were the ion exchange columns regenerated?

Answer: The IR-4 column was regenerated with ammonium hydroxide because if sodium carbonate was used great difficulty was encountered in removing the sodium ions.

Question: How was the capacity of the column determined?

Answer: By an experimental trial with the hydrolyzate mixture, which was then removed and put onto a fresh column. The capacity of the large column is about 10 g. of disaccharide and dextrin mixture calculated as maltose hydrate.

Question: How much protein is not removed by treatment with basic lead acetate?

Answer: There should be left only amino acids, which are probably removed on the ion exchange columns.

Question: What volumes of effluent liquid is collected in getting the various sugars off the column?

Answer: Starting with 10 liters of solution containing 166 g. of glucose equivalent:

Sugar	Volume in liters to remove sugar
Glucose	*20
Isomaltose	6
Dextrins	6-7

*This includes the original 10 liters. Dextrose appears first in about the fourth liter of effluent.

The Use of Paper Chromatography in the Determination of Glucose, Galactose, and Rhamnose in Mixtures -- W. L. Porter and Charles S. Fenske

Paper chromatography was used qualitatively to facilitate the quantitative determination of glucose, galactose, and rhamnose in hydrolyzates of glycosides from buckwheat. These sugars were present in very small amount. Chemical methods would have been too tedious and inaccurate to use for this analysis, and polarimetric methods were too insensitive and susceptible to interferences. The results of paper chromatography were confirmed by selective fermentation with microorganisms, and these fermentations combined with reducing sugar measurements were used to obtain the quantitative data.

Apparatus and Experimental Details.- Descending chromatography on sheets of paper was carried out in a wooden tank lined with stainless steel and having double-pane glass windows on each of the long sides. This tank had four troughs, a tightly fitting top that could be screwed down and which was provided with openings for filling the troughs after the tank was closed. A drain is to be installed at the bottom to facilitate washing the tank.

Whatman's No. 1 paper was used. A much-used solvent was butanol, 40 percent - ethanol, 10 percent - water, 50 percent. A mixture of butanol and water was tried, but gave poor separations.

Small papers were dried in an ordinary convection oven. Larger sheets were hung in a box provided with an electric fan and heated by light bulbs or by a resistance heater.

Variable results were obtained unless the filter paper before development was equilibrated in an atmosphere saturated with the aqueous phase of the solvent mixture at the operating temperature. The spots were put on the paper and it was allowed to hang for about 3 hours in the closed tank which contained the aqueous phase in a shallow vessel. At the desired time the solvent was added to the trough. Unexplained variations in the solvent front still occur. These appear to be related to the glass windows, and to the position of these windows with respect to the room during development.

Discussion

Question: Was the completeness of fermentation checked on paper chromatography?

Answer: Yes, if fermentation results appeared doubtful.

Question: What reagent was used for spraying?

Answer: Ammoniacal silver nitrate.

Question: What experience have you had with filter paper?

Answer: Whatman's No. 1 was used mostly. Schleicher and Schull paper No. 598 is much "faster," permitting a chromatogram to be completed in about 8 hours instead of 18 as is required for Whatman's No. 1. Dr. McCready stated that Whatman's No. 4 is equivalent to this S. and S. paper.

Question: Were any other reagents used for spraying?

Answer: No phenolic-type reagents were used. Phenylene diamine produced fluorescent spots.

Question: Was silver nitrate washed out of paper on which it had been sprayed?

Answer: No, the position of the sugar spot was circled with pencil, and it didn't matter if the paper turned dark later.

In further discussion, Dr. McCready stated that the work would be simplified if a Warburg apparatus were used instead of the microbiological fermentations. In 15 to 20 minutes 0.1 to 2 mg. of glucose can be detected with an accuracy of about 92 percent. The method is very specific.

Application of Paper Partition Chromatography to Sugars -- R. M. McCready

Partition analysis on paper sheets is a very attractive analytical tool because of the ease of operation, the small amount of time demanded of the operator, and the simple apparatus that suffices for use.

Variable Factors.- Factors which may be varied according to the requirements and objectives of the work to be done were discussed as follows:

1. Direction of solvent movement. The descending direction of movement is preferable to the ascending because it permits use of smaller paper, is more rapid, and gives a more even solvent front.

2. Solvents. The effectiveness of a solvent mixture for separating certain sugars can be predicted if the partition coefficients for these sugars in the solvent components are known. A solvent much used by the

speaker consisted of 70 percent butanol, 20 percent acetic acid, 50 percent water. At 25° this is a homogeneous solution; at lower temperatures (20°) two phases may separate; the temperature at which separation occurs may be lowered by increasing the content of butanol in the solution.

3. Drying. The length of drying time in an air oven may be 10 minutes or longer. Removal of solvents may be facilitated in some cases by humidifying the paper and heating again.

4. Reagents for spraying. The 3,5-dinitrosalicylic acid reagent was recommended in preference to ammoniacal silver nitrate because (a) the first-named reagent is more selective--the silver nitrate spray gives spots with salts or organic acids which may obscure sugar spots, (b) the dinitrosalicylic acid spray provides a clear, permanent record consisting of brown spots on a clear yellow background. The silver nitrate spray gives a background which darkens with age to much the same color as the sugar spots. To prevent this, unreacted silver nitrate must be washed from the paper after the black spots have been produced by reducing sugars.

The resorcinol-HCl spray for detecting ketoses (fructose and sucrose) requires heating only to 80° to bring out the delicately colored spots produced by the sugars against a pale lavender background. This heating is accomplished by a battery of four Fisher infra-red radiators; the effect of the heating can be watched and the heating stopped as desired.

Technique.- The paper used was Whatman's No. 1 or No. 4, in 30 to 40 cm. lengths. No. 4 paper permits faster movement of solvent than No. 1. With No. 1 paper, about 18 hours is used for development of the chromatogram. No effort is made to have the paper saturated with moisture during the runs, and the solvents used are not necessarily saturated with water. The presence of the solvent-saturated aqueous phase in the chamber during development of the chromatogram is not essential. Good separation results without taking these precautions.

Sugar spots are applied to the paper by a micro-pipette in quantities of 1 to 20 μl. (1 μl. = 1 cu. mm. = .001 ml.). From 0.5 to 200 μg. (1 μg. = .001 mg.) of sugar can be separated. If the concentration of the solution is small, spots can be applied successively at one point to build up the sugar concentration.

After the paper is developed and dried, the reagent is applied evenly as a very fine spray. A diagram was shown of an efficient spraying nozzle designed by Prof. Calvin of the University of California. This spray operated by air pressure and had a standard taper joint which permitted it to be used with various reagent mixtures contained in bottles with standard taper necks.

Two-dimensional development is not as useful for sugars as it is for amino acids. With sugars, the technique of multiple development, described in the abstract, is more useful for obtaining separations in difficult cases. Mannose and fructose can be separated by multiple development.

Application.- The composition of the non-uronide component of pure pectic acid, for which no good analytical data had ever been obtained before, was determined qualitatively and quantitatively by paper chromatography. The results support the conclusion that rhamnose, galactose, and arabinose, in the ratio of 3:2:1 are constituents of lemon pectin.

An acid hydrolyzate containing these sugars was streaked in a continuous line across a sheet of filter paper. A strip, running perpendicular to the sugar streak, was cut from the paper and a reducing power determination was made on the sugar mixture eluted from it to give an initial, total value. The main sheet of paper was developed, and a narrow strip of paper, running perpendicular to the original sugar streak, was cut out and sprayed to locate the positions of the separated sugars. The main sheet of paper which remained was then cut into strips parallel to the original sugar streak so that each strip contained an individual sugar. A sugar was extracted from each of these strips. Reducing determinations were made on the extracts, and from the extracts were crystallized galactose, rhamnose, and arabinose (as the diphenyl hydrazone).

Paper chromatography was used to prove the presence of glucose, fructose, and sucrose in citrus juice. This showed for the first time the presence of sucrose in lemon juice. Glucose, fructose and sucrose were removed from juice by fermentation. Chromatography on paper of the resultant solution showed no sugar to be present, and from this it was concluded that there was no rare or unusual sugar in the fruits examined.

Another application of paper chromatography described was that made by Prof. Calvin at the University of California. He uses the two-dimensional technique to separate products of photosynthesis which contain radioactive tracer-elements, and then lays the paper on X-ray film to get a permanent record of the results obtained.

Discussion

Question: Could continuous movement of solvent instead of the multiple-development technique be used to separate mannose and fructose?

Answer: The continuous drip method has not been tried on the sugars mentioned, but was used successfully on an organic acid that was a growth substance of uncertain molecular weight (50 to 1000). No movement resulted when the usual technique was employed with a benzene-acetic acid-water solvent. But when the solvent was allowed to drip off the end of the paper, the substance was moved down the paper and a distribution of activity curve was obtained.

Question: Have you used fluorescence to detect the position of sugar spots?

Answer: Yes. It is not necessary to use orthophenylene diamine. A spray of 5 percent alanine or glycine may be used, the paper heated to 120°, and then examined under ultra-violet light. The fluorescence obtained looks like "neon signs."

Question: Do you know any reagent that could detect non-reducing sugar derivatives, such as levoglucosan?

Answer: The detection of sucrose by resorcinol depends on preliminary hydrolysis of the sugar by the HCl in the reagent. If this acid were strong enough to hydrolyze substances such as levoglucosan, possibly the hydrolytic products could be detected by resorcinol. Substances such as the Schardinger dextrins and levoglucosan might be sprayed first with a solution of HCl, heated and then sprayed with a reagent for reducing sugars. But cellulose might interfere here.

There is need for some supporting medium other than paper (cellulose). Something like spun glass might be found to be useful. There is also the need to have adsorbents or supporting materials with high affinity for organic solvents. Cellulose benzoates are being given some consideration in this connection.

Question: How do you clarify sugar solutions prior to testing?

Answer: Normally, there is no need to do so. Sometimes ion-exchange is employed.

Dr. Porter described a simple and ingenious apparatus for paper chromatography originated and used by Dr. Block at New York University Medical School. A trough was placed on the bottom of a rectangular fish aquarium, near and parallel

to one side. Two glass rods were arranged parallel to the trough but no further than about 10 cm. from the bottom. One edge of the paper was placed in the solvent contained in the trough and the paper was laid across the two rods for support.

Paper Chromatography of Mono- and Disaccharide -- M. J. Wolf

Two distinct lines of work were reported: (a) Qualitative analyses on the carbohydrate constituents of the hull, germ, and endosperm of corn, and (b) exploratory work on the separation and identification of di- and higher saccharides from acid and enzymic hydrolyzates of dextran and other polysaccharides.

Apparatus.- The apparatus used is, for the most part, provisional, and has been improvised from materials on hand.

For descending chromatography, a Pyrex cylinder (45 cm. x 22 cm.) was employed which had a tightly-fitting rubber gasket and stainless steel top, and which contained a support made by joining the halves of a large Petri dish with a glass rod. The paper strips hang from the upper dish, which also holds the solvent. This cylinder and its contents was placed in a larger, closed glass container which served to diminish temperature changes in the inner cylinder. After development, the paper strips were placed in a stainless steel frame for drying and spraying.

For ascending chromatography, various arrangements have been made of two glass cylinders, 18 cm. x 6 cm., and 18 cm. x 9 cm. in size. Temperature changes in the smaller cylinder, in which the paper cylinder is placed, are diminished by placing this in the larger glass cylinder and covering this in turn with corrugated paper. Good results have been obtained by placing the solvent in the bottom of the larger glass cylinder, standing the paper cylinder containing the sugar spots in the solvent, and inverting the smaller glass cylinder over the paper. The inverted smaller glass cylinder rests on a triangular support made of glass rod which was placed on the bottom of the larger cylinder. The outer glass cylinder is closed at the top with sheet rubber.

Experimental Details.- In order to minimize irregularity in solvent front, which has been as marked in the descending as in the ascending method, attempts have been made to prevent variation in temperature during development of chromatograms, and to carry out the development in an atmosphere which is saturated with respect to both aqueous and solvent phases at the operating temperature. Because variations in R_F values have not been eliminated, identification of sugars in unknown

mixtures has been based on results of two or three separate runs in two or three separate solvent mixtures, and on results obtained by running known sugars close to unknowns on the paper and by actually placing a suitably chosen known sugar on the same spot with an unknown mixture.

It was determined experimentally that the optimum composition for the 3,5-dinitrosalicylic acid reagent for spraying was: 3,5-dinitrosalicylic acid 0.5 percent, and sodium hydroxide 4 percent.

Open platinum loops were calibrated and used for placing sugar spots on the paper.

Some exploratory work was reported on the use of solvent mixtures containing pyridine in butanol or in collidine. With increasing concentration of pyridine, increasingly high R_F values were obtained along with increasingly poor separation of sugars.

Discussion

Dr. McCready pointed out that solvents giving high R_F values often also give diffuse spots. A solvent mixture which he had found moved rapidly on the paper but gave rather low R_F values contained ethylacetate, ethanol, acetic acid and water.

During this discussion it was brought out that a fresh solvent mixture should be used for each run.

Dr. McCready stated that he had made a tabulation of R_F values for a number of sugars in various solvents, and that he would give this information to anyone who requested it.

Some Principles of Countercurrent Distribution -- H. J. Dutton

Following the report given by Dr. Dutton, Mr. Rist raised the question as to whether the Craig apparatus had distinct advantages over the partition chromatographic method. Dr. Dutton explained that the selection of the method will depend upon various conditions and the objective of the research. This discussion was in reference to the comparison Dr. Dutton had made in a table showing his selection of average data on the different procedures.

Characteristics of fractionation procedures

Apparatus	Theoretical plates	Time of heating
Rossini rotary	60	28 hours
Column, Podbilniach	50-70	24 hours
Simple pot	1	1 hour
Molecular, falling film	.75	200 seconds
Molecular, centrifugal (CMS-5)	1	0.2 seconds
Adsorption or partition chromatogram	900 - several thousands	---
Countercurrent column	1.4-29	---
Craig countercurrent distribution apparatus	25 to approx. 300*	---

*By withdrawal principle.

The value of the partition chromatographic method lies in the fact that many hundreds or perhaps a thousand plates are available for effective separation when a suitable support and solvent combination are found. The method is limited only to those solutes which are more soluble in the stationary than the moving phase. In the case of the phosphatides where partition chromatography failed because of the lack of suitable immiscible solvent pairs, the Craig countercurrent distribution apparatus succeeded in effecting a separation.

Mr. Earle inquired concerning disadvantages in the use of the Craig apparatus and Dr. Dutton replied that limitations due to emulsions and non-ideal behavior would be illustrated in subsequent papers.

Mr. Schwab inquired how one would determine the number of theoretical plates on a column. Dr. Dutton indicated that there are theoretical objections to such calculations. However, it is possible to estimate the number of plates from degree of separation measurements as described by Martin and Synge's paper (the reader is referred to Biochem. J. 35, 1358 (1941).

Dr. Jackson inquired whether it would be possible to develop a countercurrent adsorption technique. Dr. Dutton cited an instance in which light hydrocarbon gases are fractionated

by a moving column of carbon. Stationary bands of adsorbed gases were developed by the adjustment of rates of countercurrent flow for gas and adsorbent (Trans. Amer. Inst. Chem. Eng. 42, 665 (1946).

Dr. Benedict commented that a strong point in favor of the Craig apparatus is that one may be able to use buffers as an aid in the fractionation.

The reader is referred to J. Biol. Chem. 168, 687 (1947) for further discussion on the theory of countercurrent distribution.

Countercurrent Distribution Studies on Chlorophyll and Carotene Pigments -- Catherine R. Lancaster

Upon conclusion of Mrs. Lancaster's presentation of her paper, Dr. Martin asked if unknown substances could be separated by use of the Craig apparatus without too much preliminary chemical identification. Dr. Dutton expressed the opinion that the weight curve is of value regardless of whether substances do or do not behave ideally. However, care should be taken in interpreting results. In general, a determination of the partition coefficient for an unknown mixture in various solvent pairs is a desirable preliminary to countercurrent separation.

Dr. McCready was concerned with the attainment of equilibrium in the tubes of the Craig apparatus during the "flipping" procedure and asked how determination of equilibrium was made. Dr. Dutton referred to a publication, "Distribution Studies X. Attainment of Equilibrium," by Guy F. Barry, Yoshio Sato, and Lyman C. Craig, J. Biol. Chem. 174, 209 (1948), in which a method for such determination is described. The ratio of concentrations in the upper and lower phases is calculated for an increasing number of inversions. When the ratio ceases to change with increasing inversions, equilibrium has been reached. Mrs. Lancaster pointed out that a glass tube containing the solvents and solutes as in the first tube of the apparatus is fastened to the frame of the instrument and serves to indicate when separation of the phases is complete.

Application of the Craig Apparatus to Soybean Phosphatides Fractionation Problems -- C. R. Scholfield

At the close of the talk, Mr. Rist inquired as to what sugars are present in the phosphatides. Mr. Scholfield replied that although other sugars are present, the sugar was calculated as galactose. Mr. Scholfield concurred that the removal of sugars was desirable before countercurrent distribution. He also stated that the sugars were hydrolyzed with dilute sulfuric

acid, and determined by a modified Shaffer-Hartmann method. Mr. Beal inquired concerning fatty acids in relation to the structure in the phosphatide molecule. It was suggested that the observed solubility differences of inositol phosphatides might be a function of the number and kinds of fatty acids.

Comparison of Physical Methods of Fractionating Soybean Oil -- H. J. Dutton

The limitations of adsorption analysis are well known. Loss of material occurs on the column; the choice of adsorbents and solvents is largely empirical; and reversals in the order of adsorption of components may occur with change in adsorbent or solvents. Despite useful theoretical descriptions of adsorption analysis, it remains more of an art than a science.

In countercurrent distribution, probable results of a desired separation in many cases can be predicted with reasonable accuracy through preliminary investigation of the partition coefficients of solvent pairs. It is recognized that certain inherent limitations restrict the application of the countercurrent distribution apparatus. First, it is necessary to find a pair of immiscible solvents in each of which the solutes to be separated are at least partially soluble. For example, the large class of unmodified carbohydrate materials are generally excluded from Craig countercurrent distribution studies because of their extremely high differential solubility in polar solvents and practical insolubility in non-polar solvents. The tendencies of some solvents to form stable emulsions, the cost of the countercurrent equipment, the relatively small number of plates available are also included in the limitation of the technique.

Partition chromatographic methods have distinct advantages over the Craig apparatus in the large number of plates available and advantages over adsorption analysis in the low losses occurring on the column. Theory applicable to the Craig countercurrent distribution has also been extended to describe partition chromatography. With regard to borderline cases where adsorption or partition processes may occur, it was suggested that the fundamental phenomenon, whether adsorption or partition might be differentiated by the shape of the elution curve--adsorption processes being characterized by a sharp frontal boundary and trailing rear boundary and partition processes being characterized by a normal frequency distribution type curve.

Solubility limitations are even more restrictive in partition chromatography than in the Craig countercurrent distribution procedure. Not only must partition coefficients fall within

specified ranges but the more polar phase is (with few exceptions) the non-mobile phase. This restriction limits the applicability of partition chromatography to polar compounds. For example, partition chromatographic methods are generally inapplicable to the non-polar glycerides.

In conclusion, it appears that no broad generalizations can be made concerning which is the "best" method. The selection of a technique to be used will depend upon the objective of the work and upon the specific solutes to be separated.

Separation and Estimation of C_2 to C_{20} Fatty Acids in Mixtures -- W. L. Porter

After the presentation of the talk, Dr. Dutton inquired whether there was a reappearance of the blue of the indicator dye when the fatty acid bands had passed. Dr. Porter informed the group that the blue-colored chromatogram is changed to red at the location of the fatty acids, and there is a subsequent change of the red color to the blue again after the passage of the acids. The dye ($R\text{-}NH_4$), which has this particularly desirable characteristic of not being eluted during the formation of the chromatogram, is 3:6-disulpho-3-naphthalene azo-N-phenyl-α-naphthylamine (Liddel and Rydon, Biochem. J. 38, 68 (1944)).

Application of Chromatography to the Separation of Miscellaneous Compounds -- W. L. Porter

After the close of the talk, a question was raised as to whether quartz glass columns were necessary in the inspection of the chromatogram under ultra-violet light. Dr. Porter answered that Pyrex columns are generally adequate and that on occasions filters placed at the light source may be of help in the use of the ultra-violet light. In the separation of certain colorless compounds it was found that a zinc sulfide-zinc silicate adsorbent mixture is of value in following chromatographic separations. Dr. Porter further cited recent work with paper chromatography in which a photocell system was utilized to follow the separation in a chromatogram. Dr. Dutton referred to the Claesson type of refractometer which is of value in the analysis of the liquid chromatogram. The speaker disclosed that the intensity of ninhydrin spots could be increased by steam treatment and subsequent drying.

Mr. Rist inquired concerning the mechanism of the artificial browning action in synthetic maple sirup. Dr. Porter indicated that the General Foods Company had patented a reaction between glucose and α-amino-butyric acid which gives a brown color and maple flavor to sugar sirup. Dr. Porter stated that there

apparently is a relationship between color and flavor. By simply boiling the maple sirup, the desired degree of brown color may be obtained in vacuum-dried maple sirup. A question was raised concerning the quantitative paper chromatography of amino acids. Dr. Porter said that the amino acids may be determined by measuring the light reflectance from the ninhydrin spot and the area of the spot, the error being within 10 percent.

Applications of Chromatography to Separations of Minor Components of Fats and Oils -- C. E. Swift

A red oil concentrate (10 gms.) from 2,800 gms. of peanut oil was dissolved in 100 ml. of petroleum naphtha and placed on a slurry packed alumina column 7 cm. in diameter and 30 cm. high. Petroleum naphtha (700 ml.) was used to develop the column. A colored band containing 3 grams of material remained on the column and was removed by extrusion and elution of the adsorbent. A portion of this material (1.5 gms.) was readsorbed, giving 4 bands which were separated manually and which contained two pro-oxidants and two anti-oxidants.

Following Mr. Swift's talk, Dr. Jackson asked if a column had ever been used for the continuous collection of a material. Dr. Cowan mentioned that some small-scale work of that type had been done at the Valley Vitamins, Inc. plant at McAllen, Texas.

Dr. Evans inquired if precautions were taken to protect the unsaturated aldehydes from oxidation during columnar fractionation. Mr. Swift explained that oxidation does occur if the aldehydes are readsorbed several times but that in the work described the oxidation was so slight that precautions were not used.

Fractionation of Amino Acids Using Ion Exchange Resins -- R. M. McCready

A brief review was given of the types of exchange materials as exemplified by the Amberlite resins. These probably cover all the types known in the United States. In Europe, corresponding materials are produced by I. G. Farben and are called "Wolfatites."

Resin	Description	Approximate capacity millequivalents/gm.
	Cation Exchangers	
I R-100	Analytical grade, sulfonic acid type	2, cation
I R-120	High capacity; bead-form results in rapid flow; sulfonic acid type, in part	8, cation
I R-C-50	Carboxyl groups	2, cation
	Anion Exchangers	
I R-4B	Analytical grade; weakly basic amine groups	8, HCl
I R-400	Behaves like solid NaOH. Anions can be removed even from mildly alkaline solutions. (This material is very difficult to obtain.)	

A big difficulty in use of these exchangers has been that the cation exchangers have much lower capacity than the anion exchangers.

VII. CONCLUSIONS

An intangible but very important result of the Seminar was the inspiration derived by those privileged to attend. More concretely, the participants obtained not only a broad picture of the applicability of the methods but also an understanding of certain limitations, all of which should prove of value. This information will allow a more efficient attack upon future problems.

For workers who did not attend the Seminar, the following tabulation is especially presented to indicate the general applicability of the methods by substance class. A scanning of this summary will indicate where consideration should be given to the use of chromatography or countercurrent distribution. The success achieved by these tools points to the conclusion that serious consideration should be given their use in future work.

PROBLEM	PROCEDURE FOR SOLUTION
Carbohydrates	
1. Quantitative fractionation for isolation on a large scale.	Adsorption on carbon-Celite and displacement-development.
2. Qualitative and quantitative resolution of mixtures on a micro-scale.	Partition analysis on paper.
3. Proof of identity on a micro-scale.	Partition analysis on paper.
4. Exploratory resolution on a small scale.	Partition analysis on paper.
Antibiotics and Biologically Active Substances	
1. Resolution on a small or industrial scale.	Adsorption, elution, or extrusion analysis.
2. Test of purity or homogeneity.	Adsorption, elution analysis. Craig apparatus.
Vegetable Oils	
1. Quantitative fractionation.	Partition analysis by countercurrent distribution in the Craig apparatus.
2. Qualitative fractionation of minor components.	Adsorption on alumina or silicic acid, extrusion analysis.

PROBLEM	PROCEDURE FOR SOLUTION
Vegetable Oils (Cont'd.)	
3. Fractionation of products of auto-oxidation. Removal of trace constituents.	Adsorption on alumina.
Plant Pigments	
1. Fractionation, purification, and isolation on a small scale.	(a) Partition analysis by countercurrent distribution in the Craig apparatus. (b) Adsorption on starch, inulin, sucrose, Porocel, activated magnesia, Florosil, silica, calcium acid phosphate; elution analysis.
2. Concentration and purification when present in very small amount in a vegetable oil.	Adsorption on alumina or silicic acid, extrusion analysis.
Phosphatides	
1. Resolution of mixtures.	Partition analysis by countercurrent distribution in the Craig apparatus.
Fatty Acids	
1. Quantitative fractionation, isolation, and purification.	Partition analysis (columnar) on Mallinckrodt silicic acid.
2. Fractionation of C_1-C_5 acids.	Adsorption on silica.
3. Preparation and purification of methyl esters of fatty acids.	Adsorption on silica.
Sterols, Tocopherols	
1. Fractionation.	Adsorption on inulin and silica.

PROBLEM	PROCEDURE FOR SOLUTION
Sterols, Tocopherols (Cont'd.)	
2. Fractionation, isolation, and quantitative determination.	Adsorption on Hiflo Supercel and activated magnesia; elution analysis.
Vitamins and Carotenes	
1. Fractionation, isolation, and quantitative determination.	Adsorption on Hiflo Supercel and activated magnesia and Florosil; elution analysis.
Plant Alkaloids	
1. Fractionation.	Adsorption on silicic acid, elution analysis.
Amino Acids	
1. Fractionation on a small scale.	Adsorption on carbon and ion-exchange materials, elution analysis.
2. Fractionation on a micro-scale, proof of identity.	Partition analysis on paper.
Miscellaneous Compounds	
1. Purification of hydrocarbon solvents for spectroscopy, of resin acids, and of soaps used in polymerization of rubber.	Adsorption on substances such as silica gel and Fuller's earth.
2. Decomposition of polynuclear aromatic picrates for recovery of the aromatics.	Adsorption on alumina.
3. Concentration of piperonyl butoxide (insecticide) from very dilute solution.	Adsorption on silica.
4. Criterion of purity of lactic acid.	Partition analysis (columnar) on Mallinckrodt's silicic acid.
5. Fractionation of alcohols.	Adsorption of 3,5-dinitrobenzoates on silicic acid.
6. Fractionation and isolation of carbonyl compounds.	Adsorption of 2,4-dinitrophenylhydrazones on Bentonite, extrusion analysis.

VIII. RECOMMENDATIONS

1. In order that the benefits derived in this Seminar be continued, it was recommended that provision be made for the exchange of developments in this field through some form of correspondence. The periodic exchange of new developments by the several agencies of the Bureau should be made at intervals of not less than three months. In this connection it was felt that immediate knowledge of newly developed applications, techniques, methods, and apparatus would be of value to other workers, and that notice of such developments should not have to await their publication in manuscript form.

   If this recommendation is approved, it is suggested that a brief form of abstracting, according to the form given under Part V, "Classification of Chromatographic Studies," be adopted. It is possible that such a periodic list of abstracts could be distributed by attaching it as an addendum to the Laboratory and Bureau weekly newsletters. The first addendum should also contain an up-to-date list of references to the issuing Laboratory's publications in which chromatography was applied.

2. It was suggested that similar intra-Bureau meetings could be held with profit to give special attention to such subjects as analytical methods, microbiological methods, determination of amino acids, radioactivity, and the like.